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Intraspecific variations on thermal susceptibility in the predatory mite *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae): Responding to long-term heat acclimations and frequent heat hardenings

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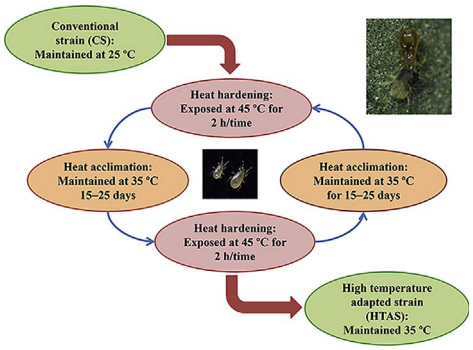
GRAPHICAL ABSTRACT


Conventional strain (CS): Maintained at 25 °C
Heat hardening: Exposed at 45 °C for 2 h/time
Heat acclimation: Maintained at 35 °C 15–25 days
Heat acclimation: Maintained at 35 °C for 15–25 days
Heat hardening: Exposed at 45 °C for 2 h/time
High temperature adapted strain (HTAS): Maintained 35 °C

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ABSTRACT

Biological control of spider mites using phytoseiid mites is frequently being disrupted by high temperatures, which results in an important and urgent research topic. We hypothesized that this scenario could be ameliorated if the thermal tolerance of a certain phytoseiid mite is enhanced. In this study, a high temperature adapted strain (HTAS) of the predatory mite *Neoseiulus barkeri* Hughes was selected from its conventional strain (CS) via a long-term heat acclimation (maintained at 35 °C) and frequent heat hardenings (exposed at 45 °C for 2 h every 15–25 days) over multiple generations. As expected, heat acclimation greatly improved the survival probabilities of *N. barkeri* when a single heat stress event occurred, with a median lethal time (e.g., at 45 °C) calculated as 15.2 h for HTAS females and 1.9 h for CS ones, respectively. After the heat acclimatory, HTAS showed a faster growth and developmental rate, and a higher immature survival rate than CS did; a significant reduced total fecundity rather than longevity was observed at 35 °C; a shifting from larger females to smaller males was observed (about 21% reduced in female ratio). Meanwhile, single heat stress event (e.g., exposed at 42 °C for 4 h) had a detrimental effect on the reproductive traits of the newly-emerged females, particularly obvious for mites from CS colony, by delaying the onset of oviposition and reducing reproductive output. This is the first experimental demonstration that the artificially heat acclimatory can shape the intraspecific variations on thermal susceptibility in a phytoseiid mite. It is suggested that HTAS *N. barkeri* might be a promising strain for the

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biological control of the high temperature favored spider mites, and more field and greenhouse trials should be evaluated in the future.

1. Introduction

Phytoseiid mites (Acari: Phytoseiidae) are effective natural enemies and important biological control agents for spider mites (McMurtry et al., 2013). In the field conditions, both phytoseiid mites and spider mites seldom live in fulfillment of all their biotic and abiotic requirements. Rather, they have to face a wide range of environmental stresses, e.g., insecticides or acaricides (Beers et al., 2016; Li et al., 2017a; Mills et al., 2016), heat or cold stress (Coombs and Bale, 2013; Jensen et al., 2017), drought stress (Ferrero et al., 2010; Montserrat et al., 2013a), ultraviolet-B (Tachi and Osakabe, 2014; Tian et al., 2017), and biotic stressors of food supply shortages (Beltrà et al., 2017; Gotoh and Tsuchiya, 2009) and pathogenic microorganisms (Bjørnson, 2008; Wu et al., 2015). As ectotherms, temperature-mediated shifts in phenotypic plasticity often vary with trophic levels. High temperature and dry conditions favore *Tetranychus* mite populations rather than phytoseiid mites (Stavrinides et al., 2010; Stavrinides and Mills, 2011), which could cause a direct biological control disruption or failure (Guzmán et al., 2016; Montserrat et al., 2013b). Moreover, the frequency and magnitude of extreme high temperature events are predicted to increase with the intensifying global warming (IPCC, 2014). Thus, the appropriate biological control strategies should be reconsidered urgently under climate warming, such as selecting for heat and/or dry resistant phytoseiid mites strains (Ghazy et al., 2016; Walzer et al., 2007; Zhang et al., 2016).

High temperature and low humidity are the main environmental factors that affect predatory mites performances in agro-ecosystems. The dry-adapted strains of *N. californicus* have been screened by Walzer et al. (2007) and its biological control trials against spider mites were also satisfactorily evaluated under hot and dry conditions (Palevsky et al., 2008; Weintraub and Palevsky, 2008). In fact, humidity in the leaf boundary layer is to be some extent higher than the atmospheric humidity. Thus, humidity might have some limited effects on phytoseiid mites (Ferro and Southwick, 1984; Zundel et al., 2007). We suspected high temperature is therefore perhaps to be the most important stressor affecting the biological and physiological parameters of phytoseiid mites.

High temperature was considered as an important selective agent causing intraspecific variations. Potential adaptations that organisms respond to stressors may concern behavioral and physiological strategies (Hoffmann et al., 2003), such as by avoiding stress, changing in growth and reproduction, or expressing of heat shock proteins and inducing enhanced antioxidants (Colinet et al., 2013; Jafari et al., 2010; Zhang et al., 2014). These behavioral and physiological changes that enable organisms to enhance thermal tolerance are usually referred to as acclimation or hardening (Hoffmann et al., 2003; Loeschcke and Sørensen, 2005). However, these adaptations in natural enemies are likely to be inadequate to counter the speed and magnitude of stressful conditions. In this study, we hypothesized that the scenario where biological controls of spider mites using phytoseiid mites are being frequently disrupted by high temperatures could be ameliorated artificially via long-term heat acclimations and frequent heat hardenings so as to enhance thermal tolerance in predators, which underlies a considerable intraspecific variability allowing the screening for a thermally tolerant strain. We considered the polyphagous predatory mite *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) as an ideal species for such a screening due to their widely distributions and applications in China (Niu et al., 2014), excellently biological performance (Li et al., 2017b,c), and well-known thermal plasticity in life history (Jafari et al., 2012, 2010; Xia et al., 2012). In fact, the commercial strains of *N. barkeri* are automatically reared and produced under a most appropriate temperature range. However, its real biological control efficiency against various pest mites in agro-ecosystems often shows a serious shrink due to their poor adaptability in field conditions. As a result, the thermal tolerance of this predatory mite is needed to be strengthened.

The objective of this study was to investigate the heat acclimatory and the consequently thermal susceptibility of the predatory mite *N. barkeri* under the high temperature stressful conditions. One was the conventional strain (CS, reared at 25 °C), and another was the high temperature adapted strain (HTAS, reared at 35 °C) that was originated from the former strain by heat acclimations and hardenings for quite a long period. Previous studies showed that heat stress not only threaten immediate survival in some insects and mites but also modify their population dynamics via impacting on life history traits such as fecundity, longevity and/or egg hatching (Yang et al., 2014; Yuan et al., 2015; Zhang et al., 2013). Moreover, heat stress events in summer are expected to become more frequently than ever. How such events differently affect CS and HTAS survival and reproduction remains unclear. In the present study, following questions were therefore considered, (1) how long-term heat acclimations and frequent heat hardenings affect life history traits of *N. barkeri* ? (2) do the effects of heat stress on adult survival vary between CS and HTAS ? (3) can a single heat stress event affect the reproductive traits in either strain or both ? To answer these questions, we accessed the heat mortality of adult females exposed at 38, 42 and 45 °C for a series of exposure durations. Heat stress temperatures of 38 and 42 °C, as well as exposure durations of 2 and 4 h were eventually selected to further study the heat effects on life history traits.

2. Materials and methods

2.1. Test mites and heat acclimation

The stock culture of *N. barkeri* was kindly shared by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. *N. barkeri* was fed on flour mites *Aleuroglyphus ovatus* in 4 L translucent plastic rearing boxes closed with a lid where a 16 cm^2 fine mesh (45 μm opening) was made to pass air. They were held at constant 25 ± 1 °C, with 70–80% RH and a L:D = 14:10 h photoperiod in a climate-controlled room. Mites maintained at 25 °C were subjectively considered as the conventional strain (CS).

In order to obtain HTAS of *N. barkeri*, we initiated the heat acclimation with approximately 10, 000 mites from CS colony. Heat acclimatory had two main processes, including short-term heat exposures (heat hardening) and long-term high temperature exposures (heat acclimation). Specifically, in April 2012, CS *N. barkeri* was firstly heat hardened at 45 °C for 2 h in a programmable temperature controller (Ningbo Southeast Instrument Co. Ltd., RDN-300B-4, Ningbo, China). The surviving mites were then maintained at a heat acclimation condition of 35 ± 1 °C and 75 ± 5% RH in another controller. When the population of *N. barkeri* reached to the maximum value (about 15–25 days after the last heat hardening), the next heat hardening process was performed followed by putting them back to acclimation condition again. Fresh wheat bran was updated every 6–8 days by mixing 30% old wheat bran (calculated by volume) containing all mites with 70% fresh wheat bran. HTAS had been heat acclimated for at least 110 generations at 35 °C and heat hardened for 25 times before our experiments.

2.2. Adult female immediate heat mortality

An iron mesh (250 μm opening) was used to separate predatory mites from their wheat bran colony. One hundred adult females were firstly collected onto a plastic film (35 mm diameter) floated on a water soaked sponge (45 mm diameter) in Petri dishes (60 mm diameter). An arched piece of filter paper (20 mm × 10 mm) was then placed in the center of the plastic film. Gradually, mites began climbing the filter paper and gathered beneath the arched paper. By using a pointed tweezers, the filter paper along with 50–70 mites were transferred into a reprocessed 1.5-mL centrifuge tube which was similar to the tube used and described by Ghazy et al. (2014). Specifically, each tube had a ventilator (6 mm diameter) with a gas-permeable filter (45 μm opening) on the lid. To make the mites fallen off from the filter paper and fallen into the tube, three to five strong beat were made on the outer tube. Then, the filter paper was taken out immediately and the tube was enclosed.

To assess the adult female immediate heat mortality, tubes with CS or HTAS adults were exposed at each target temperature including 38, 42 and 45 °C in a programmable temperature controller. The duration of each temperature treatment was 2, 4, 8, 12, 16 and 24 h. The exposure durations of 6, 10 and 14 h were additionally included when exposed at 45 °C. Mites maintained at 25 °C served as a control. The stressed mites were immediately placed at 25 °C till 24 h, followed by pouring all mites in each tube onto a piece of A4 paper to check for mortality. Mites that were unable to move a distance of 1 mm were considered dead when touched with a fine camel's hair brush. Each treatment was replicated at least five times.

2.3. Effect of heat acclimation temperature on immature development and reproduction

Immature development studies of CS and HTAS mites were investigated at 25 °C and 35 °C, respectively. Prior to an experiment, 100 Petri dishes (60 mm diameter) were prepared. In each dish, a cowpea leaf arena (25 mm diameter; infected with the all stages of *Tetranychus urticae* at least for 3 days) was placed upside down on water-saturated absorbent cotton (40 mm diameter) and round sponge (55 mm diameter and 2 mm thickness) (Zhang et al., 2016). One hundred even-aged eggs (< 6 h) from the CS or HTAS stock cultures were individually transferred onto each leaf arena, and leaf arenas were then transferred to a programmable temperature controller that was set at each desired temperature, with 85 ± 5% RH and a photoperiod of 14:10 (L:D) h. To determine the duration of the immature stages (egg, larva, protonymph and deutonymph) of *N. barkeri* for each strain, inspections were carried out once every 6 h under a stereomicroscope until the predators reached adulthood.

Fifty newly emerged adult females from each temperature regime were transferred into a new leaf arena to assess the survival and number of eggs deposited in daily intervals until the predators died. Mites escaped from the leaf arena were excluded from the analyses. Daily observations were made in an air-conditioned room around 25 °C. In addition, to minimize the escape rate of the predators during our experiments, a piece of wheat bran was added in the middle of leaf arena, served as shelter, as well as oviposition site for predators. The predatory mites were transferred to new arenas every 6 or 8 days.

2.4. Effect of heat stress on reproduction

To investigate how a single heat stress affects reproduction for CS and HTAS *N. barkeri* differently, newly-emerged females (1–2 days old)

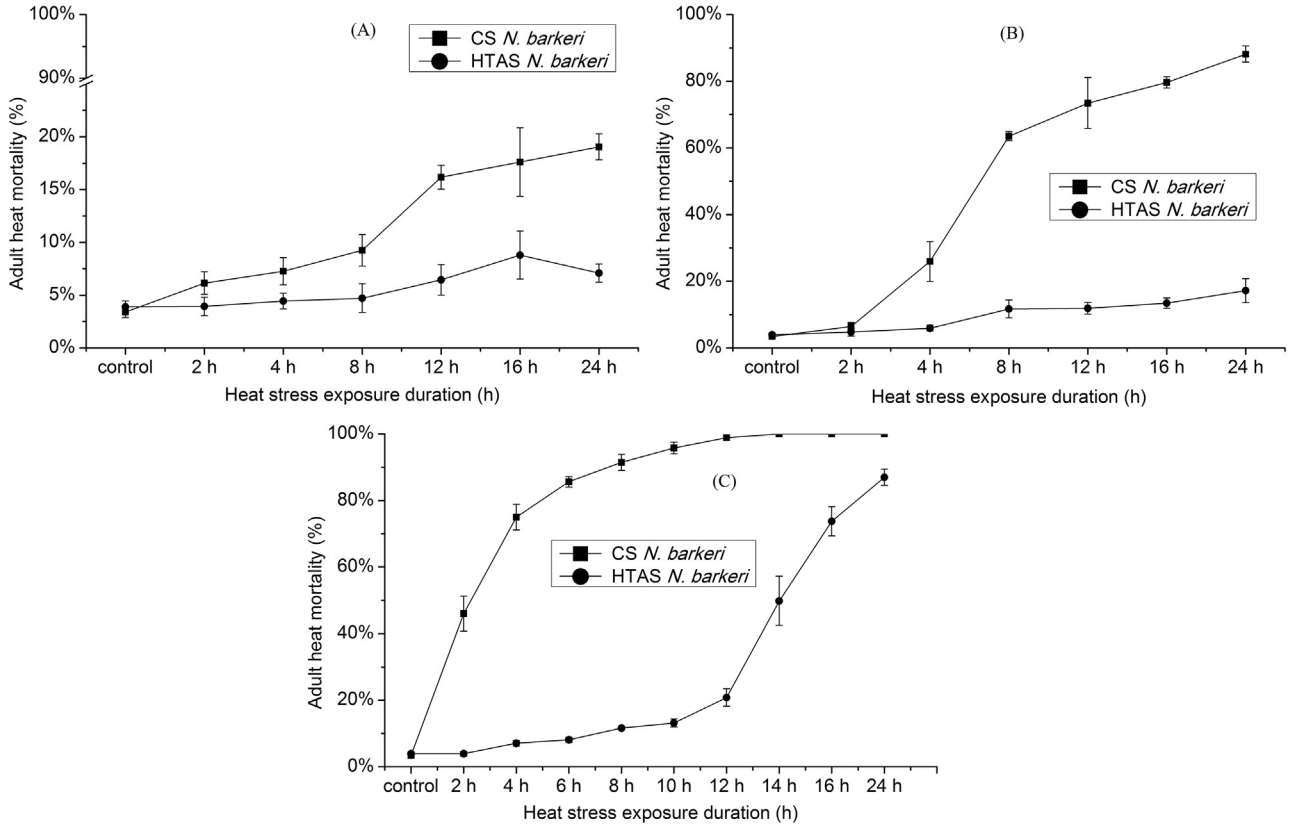

Fig. 1. Effects of heat stress on adult female heat mortality of CS and HTAS *Neoseiulus barkeri*; Each value represents the Mean ± SE; (A)–(C): exposed at 38, 42 and 45 °C, respectively; Mite maintained at 25 °C served as control.

obtained from each stock cultures were subjected to 38 and 42 °C for 2 and 4 h, respectively. Each stress temperature-duration combination was consisted of 70–80 individually replications. After heat exposure, an even-aged male (unstressed) was added into leaf arena for copulation. Mites reared at 25 °C served as control. Survival and newly deposited eggs were recorded daily until predators died.

2.5. Statistical analyses

Adult female immediate heat mortality (arcsine square root transformed), longevity, fecundity of the heat treated adult females over strains, exposure temperatures, and exposure durations were subjected to a three-way ANOVA by using the general linear model procedure of SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA), and mean differences were separated by least significant difference (*LSD*) test when significant *F*-values were obtained ($P < 0.05$). Heat mortality (arcsine square root transformed), longevity and fecundity of the heat treated adult females were all analyzed using one-way ANOVA, and significant differences between means were tested by least significant difference (*LSD*) test when significant *F*-values were obtained ($P < 0.05$). Student's *t*-tests ($P < 0.05$) were also used to compare mean differences between CS and HTAS. Survival analysis was performed with Kaplan-Meier method followed by the log rank (Mantel-Cox) test for pair-wise comparisons between the CS and HTAS. The median lethal time (LT_{50}) and lethal time (LT_{95}) were estimated by using probit analysis (SPSS 16.0).

3. Results

3.1. Adult female immediate heat mortality

Three-way ANOVA showed that the adult heat mortality was significantly affected by heat acclimation ($F = 1150.874$; df = 1, 176; $P < 0.001$), stress temperatures ($F = 963.615$; df = 2, 176; $P < 0.001$) and exposure durations ($F = 150.594$; df = 8, 176; $P < 0.001$), and the interaction among three factors were also significant ($F = 19.515$; df = 10, 176; $P < 0.001$). The mortality of the HTAS mites was obviously not affected (or just marginally) by the exposure duration at 38 °C (Fig. 1A) and 42 °C (Fig. 1B), whereas the mortality was first also low at 45 °C until it increased strongly when the exposure duration was longer than 12 h (Fig. 1C). Contrary, the mortality of the CS mites was positively correlated with the exposure duration and temperature (Fig. 1).

Heat acclimatory greatly improved the survival probabilities of *N. barkeri* adult females. When exposed at 45 °C, the LT_{50} value of HTAS females was calculated as 8.0 times longer than that of CS ones, and the LT_{95} value of HTAS was also as 3.0 times longer than CS, respectively (Table 1).

3.2. Development and reproduction under heat acclimation temperature

At 25 °C, the developmental duration (egg-adult) was significantly decreased (♀: $t = 27.036$; df = 114; $P < 0.001$; ♂: $t = 19.342$; df = 67; $P < 0.001$) in HTAS mites (♀: 4.538 ± 0.047 days; ♂: 4.390 ± 0.053 days) in comparison to CS mites (♀: 6.039 ± 0.032 days; ♂: 5.875 ± 0.048 days) (Table 2). The survival rate of all immature stages was 93.0% in CS mites and 92.0% in HTAS mites, respectively (Table 2). When CS mites were maintained at 35 °C, it showed an accelerated developmental duration (♀: 4.406 ± 0.056 days; ♂: 4.411 ± 0.107 days) and an increased mortality (35.0%), whereas HTAS mites (♀: 4.027 ± 0.023 days; ♂: 3.963 ± 0.020 days) developed fast, and also had a very high survival rate (90.0%). Meanwhile, it should be noted that heat acclimation could also result in a decreased sex ratio, with female percentage of 69.5% (25 °C) and 73.8% (35 °C) in CS mites, and 55.9% (25 °C) and 57.3% (35 °C) in HTAS mites, respectively (Table 2).

The total fecundity and mean longevity of CS and HTAS *N. barkeri* are given in Table 3. At 25 °C, adult females of CS and HTAS *N. barkeri* laid an average eggs of 66.43 ± 1.89 and 65.96 ± 1.92 per female ($t = 0.171$; df = 97; $P = 0.865$), and survived a mean longevity recorded as long as 51.88 ± 1.73 days (CS) and 48.72 ± 1.48 days (HTAS) ($t = 1.390$, df = 97, $P = 0.168$), respectively (Table 3). At 35 °C, total fecundity in CS females was significantly higher than that was in HTAS females ($t = 2.154$, df = 37, $P = 0.038$), while their longevity was not statistically different from each other ($t = 1.240$, df = 37, $P = 0.223$) (Table 3).

Survival curves were significantly affected by temperature according to log rank tests (CS: $\chi^2 = 89.402$, df = 1, $P < 0.001$; HTAS: $\chi^2 = 100.895$, df = 1, $P < 0.001$). However, no significant difference was observed when CS or HTAS females maintained at the same temperature (25 °C: $\chi^2 = 2.018$, df = 1, $P = 0.155$; 35 °C: $\chi^2 = 0.524$, df = 1, $P = 0.469$) (Figs. 2 and 3).

3.3. Heat stress effects on pre-oviposition period, fecundity and longevity

Pre-oviposition period (POP) was significantly affected by heat acclimation ($F = 18.673$; df = 1, 496; $P < 0.001$), and exposure durations ($F = 13.776$; df = 1, 496; $P < 0.001$), while the effect of stress temperatures on POP was not significant ($F = 1.419$; df = 1, 496; $P = 0.234$). When maintained at 25 °C, HTAS adult females showed a significant prolonged POP in comparison to CS adults, with 5.22 ± 0.20 days and 4.05 ± 0.15 days, respectively ($t = 4.496$; df = 97; $P < 0.001$). When exposed at 38 °C for 2 and 4 h, the average POP in CS mites was significant extended by 1.22 days and 2.49 days in comparison to control ($F = 27.459$; df = 2, 137; $P < 0.001$), respectively (Fig. 4A). HTAS adults also had an extension of 0.76 days and 1.19 days ($F = 6.771$; df = 2, 166; $P < 0.001$), respectively (Fig. 4A). Similar extensions were also found at 42 °C (CS: $F = 30.785$; df = 2, 137; $P < 0.001$; HTAS: $F = 12.121$; df = 2, 153; $P < 0.001$) (Fig. 4B).

Total fecundity were significantly affected by stress temperatures ($F = 33.328$; df = 1, 496; $P < 0.001$), exposure durations ($F = 8.358$; df = 1, 496; $P = 0.004$), but not significantly different between strains ($F = 0.015$; df = 1, 496; $P = 0.903$). No significant interactions among those three factors were detected ($F = 0.209$; df = 1, 496; $P = 0.648$). Overall, high temperature had an adverse effect on fecundity of adult females. When CS mites were exposed to 38 °C for 2 and 4 h, the total fecundity distinctly decreased ($F = 4.401$; df = 2, 137; $P = 0.014$), respectively (Fig. 4C). Similar decreased fecundity were also observed at 42 °C ($F = 24.592$; df = 2, 137; $P < 0.001$) (Fig. 4D). Whereas in HTAS mites, a significant decreased total fecundity was found only when mites exposed at 42 °C for 2 and 4 h ($F = 10.106$; df = 2, 153; $P < 0.001$).

Female longevity was not significantly different between strains ($F = 2.265$; df = 1, 496; $P = 0.133$), but it was significantly affected by stress temperatures ($F = 5.812$; df = 1, 496; $P = 0.016$), and exposure durations ($F = 4.222$; df = 1, 496; $P = 0.040$). The interactions among three factors were not significant ($F = 0.107$; df = 1, 496; $P = 0.744$).

Table 1
The median lethal time (LT_{50}) and lethal time (LT_{95}) of two strains of *N. barkeri* when exposed at 42 or 45 °C.

Temperatures (°C)	Strains	Median lethal time (h) LT_{50} (95% Confidence interval)	Lethal time (h) LT_{95} (95% Confidence interval)
42	CS	8.8 (7.1–10.4)	22.9 (19.8–27.9)
	HTAS	–	–
45	CS	1.9 (1.2–2.5)	8.8 (8.2–9.6)
	HTAS	15.2 (14. 4–16.1)	26.0 (24.2–28.3)

Note: "–" indicates that the value of LT_{50} or LT_{95} cannot be calculated by using probit analysis, owing to the high survival rate in HTAS adult females when exposed at 42 °C for 0–24 h.

Table 2
Developmental duration in days (mean ± SD), survival of immature stages and sex-ratio of *Neoseiulus barkeri* when fed on *Tetranychus urticae*.

Temp.	Strain & Sex	Egg	Larva	Protonymph	Deutonymph	Egg to adult	Survival to adulthood (%)	Sex-ratio (%)
25 °C	CS ♀	**2.219 ± 0.031***	**0.945 ± 0.033***	**1.477 ± 0.036***	1.398 ± 0.033*	**6.039 ± 0.032***	92.0	69.5
	CS ♂	**2.196 ± 0.046***	0.946 ± 0.059*	**1.428 ± 0.042***	**1.303 ± 0.046***	**5.875 ± 0.048***		
	HTAS ♀	**1.980 ± 0.019**	0.548 ± 0.021	**0.981 ± 0.031**	**1.028 ± 0.016**	**4.538 ± 0.047**	93.0	55.9
	HTAS ♂	**1.914 ± 0.034**	0.536 ± 0.020	**0.914 ± 0.034**	**1.024 ± 0.017**	**4.390 ± 0.053**		
35 °C	CS ♀	1.145 ± 0.033*	0.812 ± 0.035*	1.145 ± 0.039	1.302 ± 0.035	4.406 ± 0.056*	65.0	73.8
	CS ♂	1.176 ± 0.059*	0.882 ± 0.053*	1.205 ± 0.074	1.147 ± 0.056	4.411 ± 0.107*		
	HTAS ♀	1.045 ± 0.019	0.536 ± 0.017	1.227 ± 0.033	1.218 ± 0.033	4.027 ± 0.023	90.0	57.3
	HTAS ♂	1.036 ± 0.020	0.524 ± 0.017	1.243 ± 0.039	1.158 ± 0.036	3.963 ± 0.020		

Note: Sex-ratio (percent of females); Numbers within the same column followed by asterisk "*" are significantly different between CS and HTAS in a same developmental temperature (*t*-test, $P < 0.05$); Numbers shown in bold indicate the significant difference in developmental duration between 25 °C and 35 °C within a same strain (*t*-test, $P < 0.05$).

Table 3
Effects of high temperature on fecundity and longevity of *Neoseiulus barkeri*.

Temp.	Strain	Fecundity (eggs/female)	Longevity (days)
25 °C	CS	66.43 ± 1.89	51.88 ± 1.73
	HTAS	65.96 ± 1.92	48.72 ± 1.48
35 °C	CS	23.91 ± 1.55*	22.23 ± 1.28
	HTAS	19.35 ± 1.32	19.71 ± 1.61

Note: The asterisk "*" represents significant difference between CS and HTAS in a same developmental temperature (*t*-test, $P < 0.05$).

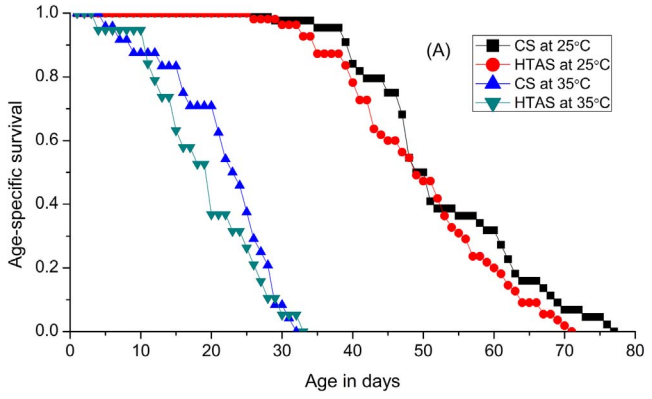

Fig. 2. Effects of high temperature (35 °C) on the age-specific survival rate (l_x) of adult female from CS and HTAS *Neoseiulus barkeri*.

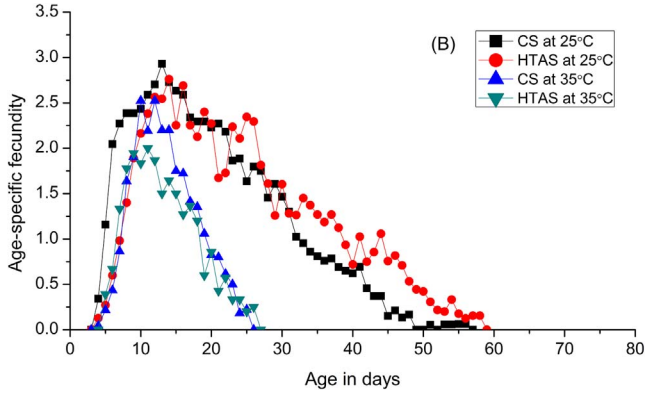

Fig. 3. Effects of high temperature (35 °C) on the age-specific fecundity (m_x) of adult females from CS and HTAS *Neoseiulus barkeri*.

When CS adult females exposed either at 38 °C ($F = 4.424$; df = 2, 137; $P = 0.014$) or 42 °C ($F = 21.311$; df = 2, 137; $P < 0.001$) for 2 and 4 h (Fig. 4E and F), female longevity was significantly decreased in comparison to control. However, the average female longevity of HTAS adult females was not affected by any exposure of 38 °C ($F = 0.412$; df = 2, 166; $P = 0.663$) or 42 °C ($F = 0.168$; df = 2, 153; $P = 0.846$) (Fig. 4E and F).

Survival curves of CS mites were significantly affected by heat stress according to log rank tests (overall: $\chi^2 = 50.290$, df = 4, $P < 0.001$), but the significant difference was not found in HTAS mites (overall: $\chi^2 = 1.877$, df = 4, $P = 0.758$) (Fig. 5). When exposed at 42 °C, survival curves differed significantly between CS and HTAS mites both for 2 h exposure ($\chi^2 = 18.069$, df = 1, $P < 0.001$) and for 4 h exposure ($\chi^2 = 17.590$, df = 1, $P < 0.001$) (Fig. 5).

4. Discussion

The present study investigated how the long-term heat acclimations (35 °C) and the frequent heat hardenings (45 °C for 2 h exposure) affected the thermal tolerance of *N. barkeri* in the laboratory conditions. Several metrics were used to assess their thermal tolerance between CS and HTAS mites, including the immediate heat mortality, immature duration, survival and reproduction. All metrics confirmed a clear pattern of the beneficial acclimation that could potentially improve the survival probabilities of *N. barkeri* during the high temperature stressful conditions.

Adult female mortality caused by heat stress was significantly lower in HTAS adults than that was observed in CS individuals (Fig. 1A–C), indicating HTAS *N. barkeri* could endure a more serious heat stress. Heat acclimation had greatly improved the survival probability up to 90% when exposed at 45 °C for 2–6 h. Adult female are considered to be the most heat-tolerant stage. Nevertheless, adult female from CS colony showed a slight mortality (< 20%) when exposed at 38 °C for 24 h. It is well known that phytoseiid mites are tiny organisms with a large surface area to volume ratio (Yoder, 1998). During heat stress, mites had no choice but to enhance transpiration and respiration to regulate their body temperatures (Gibbs, 2002; Yoder et al., 2005) or waited for the advent of death. Without liquid intake from preys, free water or atmospheric humidity to compensate for their body water content (Gaede, 1992; Walzer et al., 2007), heat stressed individuals might lose a lot of body water and eventually died, leaving a wizened body. Therefore, we suggested that the deficit in body water could be one of the possible reasons that caused massive mortality during heat stress.

Both CS and HTAS *N. barkeri* completed their growth, development, copulation and egg laying behavior successfully when maintained at a constant temperature of 35 °C, but they showed an accelerated growth and developmental rate in comparison to the CS mites at 25 °C. We suggested that this speeded growth and developmental rate within a generation might be explained by a plastic response within a generation, and therefore help *N. brakeri* reduce the exposure duration of their juveniles to heat stress. However, CS mites showed a higher mortality at immature stages in comparison to HTAS ones (Table 2). This improved the survival probabilities at high temperature in HTAS could be explained as a result of the heat acclimatory (Colinet et al., 2013). Previous study suggested that individuals maintained at high temperatures

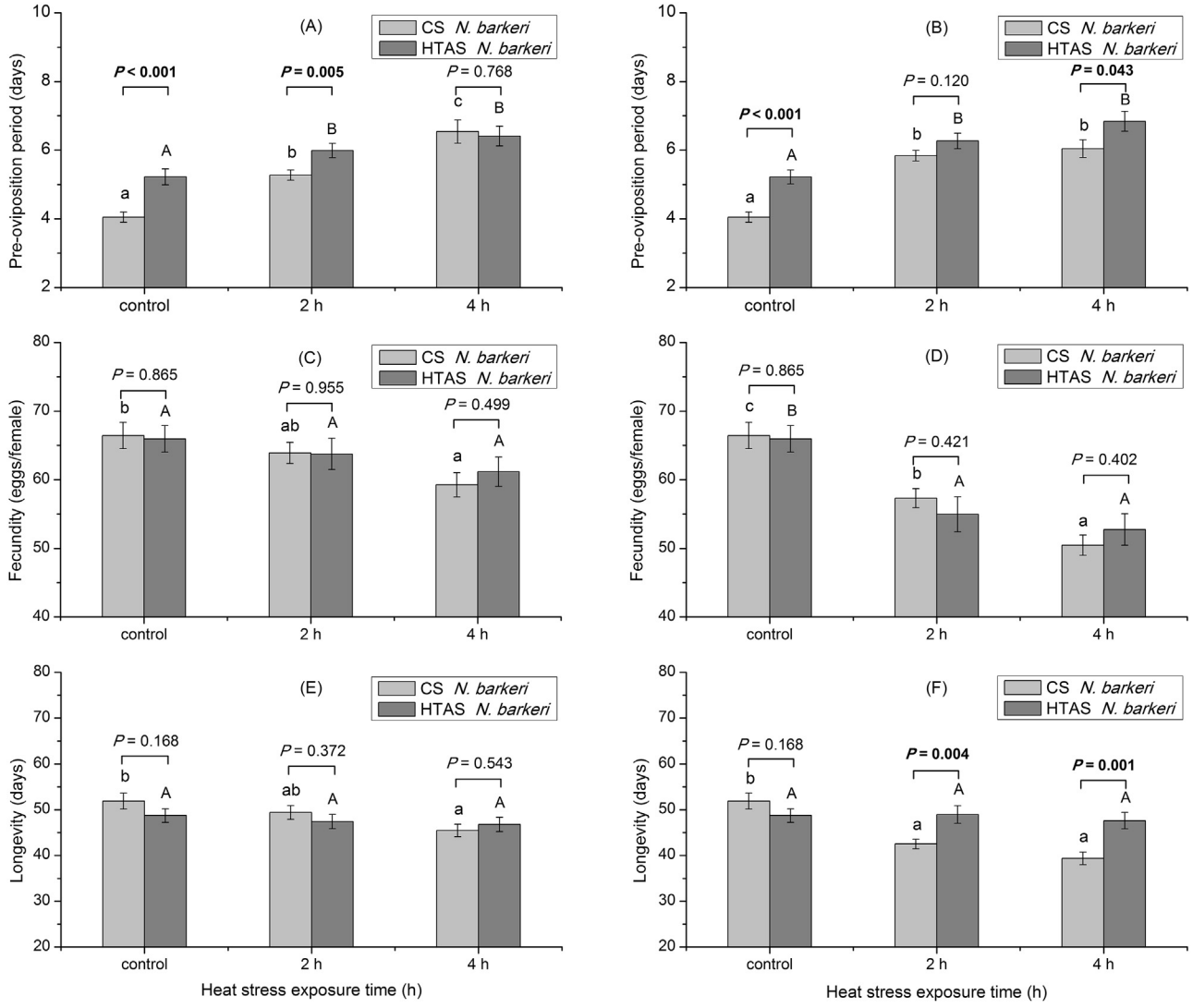

Fig. 4. Effects of heat stress on the pre-oviposition period (POP), fecundity and longevity of the adult females from CS and HTAS *Neoseiulus barkeri*; Each value represents the Mean ± SE; Different lower case letters (for CS mites) or capital letters (for HTAS mites) topped on the bar indicated the significant difference among exposure durations of 0, 2 and 4 h within each strain when the mites were exposed at each target stress temperature of 38 °C or 42 °C, $P < 0.05$, *LSD* in one-way ANOVA; *P*-value represents the significant difference in life history traits between HTAS and CS adult females, *t*-test; (A), (C) and (E): 38 °C, (B), (D) and (F): 42 °C, respectively; 25 °C served as control.

grow faster but attain a smaller body size upon maturity (Atkinson, 1994), which could reduce the area of the heat exposure and therefore protect them from the heat injury. In our study, we found the sex-ratio of HTAS was affected by heat acclimation, with a shift from larger females to smaller males. Maybe size-dependent mortality was the responsible reason, affecting more in the larger females than smaller males. Similar shifts were also found in *N. barkeri* by Xia et al. (2012) and in another predatory mite *N. californicus* by El Taj and Jung (2012), however, the heat acclamatory response was not studied in those studies.

When maintained at 35 °C, *N. barkeri* showed a significant reduced total fecundity and a shortened longevity. The reduced fitness caused by high temperature was similar to that observed by Jafari et al. (2010) and Xia et al. (2012). Although HTAS mites showed a lower total fecundity than CS mites at 35 °C, no significant difference in life span was observed between two strains. Considering the adult heat mortality, a trade-off between survival and reproduction might be existed in HTAS mites when it was held at 35 °C, which could improve their survival probability.

Previous studies reported that reproductive traits are much more sensitive to thermal stress than many other physiological traits (Nguyen et al., 2013; Zhang et al., 2013). Although heat stress within 2–4 h at 38–42 °C did not result in a directly high mortality in CS and HTAS adults (Fig. 1A and B), the stressed virgin females had a reduced total fecundity or shortened longevity with the rise of stress temperature and extension of exposure duration (Fig. 4), which indicated that the reproductive traits were negatively affected by heat stress. Similarly the reduced total fecundity and shortened longevity after heat stress were found in spider mite *Panonychus citri* (Yang et al., 2014) and predatory mite *N. californicus* (Yuan et al., 2015). Therefore, we speculated that heat stress might cause a direct delaying for egg laying in both CS and HTAS. In addition, heat stress effects on life history triats vary among trophic levels, causing a suggested disruption of biological control (Guzmán et al., 2016; Montserrat et al., 2013b). The reproductive traits were not affected by the heat stress of 38–46 °C for 2–6 h in some *Tetranychus* mites, e.g., *T. turkestani* and *T. truncates* (Yang et al., 2013), and even increased in *T. viennensis* after the heat exposure at 46 °C for 6 h (Li et al., 2010). In these studies, *Tetranychus* mites were exposed to a more serious heat stress than that *N. barkeri* had experienced in the present study, however, the reproductive performance in the laboratory conditions was enhanced for most *Tetranychus* mites and also showing a population outburst in field hot-dry conditions (Duso and Pasqualetto,

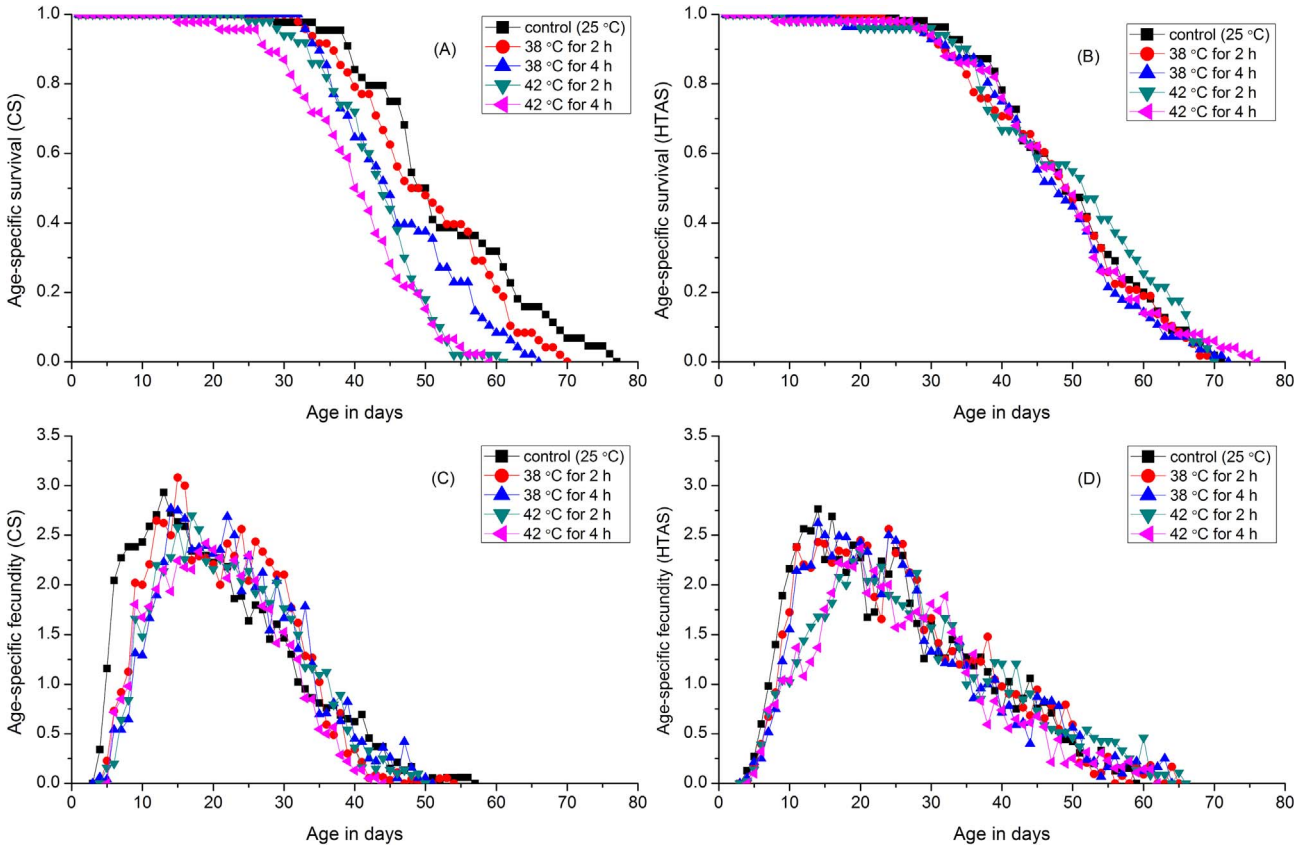

Fig. 5. Effects of heat stress on the age-specific survival rate (l_x) and age-specific fecundity (m_x) of the adult females for CS and HTAS *Neoseiulus barkeri*; (A) and (B): l_x for CS and HTAS, respectively; (C) and (D): m_x for CS and HTAS, respectively.

1993; Guzmán et al., 2016). We speculated that high temperatures and extreme hot weather events in hot summer days might cause a direct biological control disruption or failure. However, HTAS *N. barkeri* had a well-adapted performance in survival and reproduction traits particularly when exposed to heat stress, which might make up for the CS or some other phytoseiid mites deficiency when used to control those high temperature-favored spider mites.

5. Conclusion

Biological control of spider mites in hot and dry summer is a serious technical issue. Selecting high temperature adapted predatory mites might be an effective approach to ameliorating the issue. Our results demonstrated that the thermal susceptibility of *N. barkeri* was distinctly enhanced after heat acclimations and heat hardenings. These intraspecific variations could contribute to the adaptive plastic responses. We speculated that HTAS *N. barkeri* might have fully developed or innately activated some thermal tolerance factors to deal with thermal stress, such as the well-known heat shock proteins or other alternative mechanisms that need further investigations. We concluded that the HTAS *N. barkeri* might be a promising strain for biological control in its warming world, and more field trials are needed to evaluate its biological control efficiency against pest mites under hot and dry conditions.

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